Institute Report No. 320

Mutagenic Potential of Physostigmine Salicylate in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test

Suzanne E. Sebastian, BA, SPC, USA
and
John W. Harbell, PhD, MAJ, MSC

GENETIC TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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ABSTRACT

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Key Words: Mutagenicity, Genetic Toxicology, Ames Test, PHYSOSTIGMINE SALICYLATE

PREFACE

TYPE REPORT: Ames Test GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010-5425
Project Officer: LTC J. von Bredow, PhD, MSC

PROJECT/WORK UNIT/APC: 3M162734A875/308/TLEO

GLP STUDY NUMBER: 87001

STUDY DIRECTOR: MAJ John W. Harbell, PhD, MSC

PRINCIPAL INVESTIGATOR: Suzanne E. Sebastian, BA, SPC, USA

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, retired SOPs, stability and purity data on the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: PHYSOSTIGMINE SALICYLATE

INCLUSIVE STUDY DATES: 30 January 1987 - 3 April 1987

OBJECTIVE:

The objective of this study was to determine the mutagenic potential of PHYSOSTIGMINE SALICYLATE (LAIR Code TW73) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

ACKNOWLEDGMENTS

SGT Lillie D. Witcher, BS, USA, and SGT Gayle Orner, BS, USA provided research assistance. MAJ Don W. Korte, Jr., PhD, MSC provided program guidance and facilitated the conduct of the study and the publication of the final report.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 87001 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

John W Harbell 8 June 88

JOHN W. HARBELL, PhD / Date
MAJ, MS
Study Director

Suzanne E. Sebastian 24 OCTOBER 88

SUZANNE E. SEBASTIAN, BA / DATE
SPC, USA
Principal Investigator

Conrad Wheeler 27 Oct 88

CONRAD R. WHEELER, PhD / DATE
DAC
Analytical Chemist

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF

SGRD-ULZ-QA

9 December 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 87001

1. This is to certify that in relation to LAIR GLP Study 87001, the following inspections were made:

26 January 1987	-	Protocol Review
13 February 1987	-	Plate Counting (Pilot)
31 March 1987	-	Dosing (Final Assay)

2. The institute report entitled "Mutagenic Potential of Physostigmine Salicylate in the Ames Salmonella/Mammalian Microsome Mutagenicity Test," Toxicology Series 203, was audited on 24 November 1987.

Carolyn M. Lewis

CAROLYN M. LEWIS, MS
Diplomate, American Board of Toxicology
Chief, Quality Assurance

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Mutagenic Potential of PHYSOSTIGMINE SALICYLATE in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test- Sebastian and Harbell

INTRODUCTION

Soman, the primary nerve agent utilized by threat forces, is refractory to the standard antidotal therapy, atropine and pralidoxime (2-PAM), fielded by the US Army. Consequently, the highest priority has been placed on fielding a more effective treatment regimen. A regimen incorporating pyridostigmine as a prophylactic agent, combined with standard atropine/2-PAM therapy, has proven extremely effective in reducing mortality of Rhesus monkeys exposed to multilethal concentrations of soman (1). However, these animals require a prolonged period of recovery during which they are completely incapacitated. This has been attributed to the quaternary nature of pyridostigmine, which does not cross the blood-brain barrier and thus only protects the peripheral nervous system. Consequently, a tertiary carbamate, PHYSOSTIGMINE, has been proposed for the prophylactic regimen since it would protect the central nervous system in addition to the peripheral nervous system. Experimental studies support this hypothesis as animals pretreated with physostigmine before exposure to soman recover at a faster rate than animals pretreated with pyridostigmine (2,3). An enhanced rate of recovery of soldiers from a multilethal exposure to soman would produce a decided advantage in maintaining a fully functional military unit during a future conflict.

Although PHYSOSTIGMINE has been available for more than a century (4), little directed research on its mutagenic potential has been conducted. Consequently, the Division of Toxicology, Letterman Army Institute of Research, was tasked by the US Army Medical Research Institute of Chemical Defense to provide a mutagenicity profile of PHYSOSTIGMINE SALICYLATE. This report describes the results of a mutagenicity study of PHYSOSTIGMINE SALICYLATE in the Ames test.

The Ames *Salmonella*/Mammalian Microsome Mutagenicity Test is a short-term screening test that utilizes histidine auxotrophic mutant strains of *Salmonella typhimurium* to detect compounds that are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the test to increase sensitivity by simulating *in vivo* metabolic activation of the test compound. The Ames Test is an

inexpensive yet highly predictive and reliable test for detecting mutagenic activity and thus carcinogenic potential (5).

This evaluation of PHYSOSTIGMINE SALICYLATE utilizes a revision of the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (6). Two new tester strains, a frame-shift strain (TA97) and a strain carrying an ochre mutation on a multicopy plasmid (TA102), are added to the standard tester set.

Objective of the Study

The objective of this study was to determine the mutagenic potential of PHYSOSTIGMINE SALICYLATE (LAIR Code TW73) by using the revised Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

MATERIALS AND METHODS

Test Compound

Chemical Name: PHYSOSTIGMINE SALICYLATE

LAIR Code Number: TW73

Physical State: White crystalline solid

Source: Division of Experimental Therapeutics
WRAIR, Washington, D.C.
Requested by LTC von Bredow, USAMRICD

Storage: PHYSOSTIGMINE SALICYLATE was received and assigned the LAIR Code number TW73. The test compound was stored in a desiccator at -20°C until used.

Chemical Properties/Analysis: Data provided by WRAIR characterizing the chemical composition and purity of the test material are presented in Appendix A along with confirmatory analysis of the test material performed by the Division of Toxicology, LAIR (Presidio of San Francisco, CA).

Test Solvent

The positive control chemicals were dissolved in grade I dimethyl sulfoxide (lot 113F-0450) obtained from Sigma Chemical Co. (St. Louis, MO). The test compound was dissolved in glass-distilled water. The glass-distilled water used in this assay was first passed through a Technic

Model 301 Reverse Osmosis Unit (Seattle, WA), then through a Corning MP-1 Mega-Pure System glass distillation unit (Corning Glass Works, Corning, NY) (7).

Chemical Preparation

On the day of dosing, 300 mg of the test compound was measured into a sterile vial and dissolved in glass-distilled water to achieve a 5% (w/v) solution. Aliquots of this solution were used to dose the test plates.

Test Strains

Salmonella strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 obtained directly from Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory in liquid nitrogen. Quality control tests were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rates. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (8).

Test Format

PHYSOSTIGMINE SALICYLATE was evaluated for mutagenic potential according to the revised Ames method (6). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (8).

Toxicity Tests:

Toxicity tests were conducted to determine a sublethal concentration of the test substance. This toxicity level was found by using minimal glucose agar (MGA) plates, concentrations of PHYSOSTIGMINE SALICYLATE ranging from 1.6 x 10^{-3} mg/plate to 5 mg/plate, and approximately 10^{8} cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin was placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth on the plates was observed. Since the two highest doses showed a decreased number of macrocolonies (below the spontaneous rate) and an observable reduction in the density of the background lawn, the highest dose selected for the mutagenicity test was 0.2 mg/plate.

Mutagenicity Test:

The test substance was evaluated over a 1000-fold range of concentrations, decreasing from the minimum toxic level (the maximum or limit dose) by a dilution factor of 5, both with and without 0.5 ml of the S-9 microsome fraction. The S-9 (lot R-315) was purchased from Microbiological Associates, Inc. (Bethesda, MD). A standard S9 mix (4%) was used (6). After all the ingredients were added, the top agar was mixed, then overlaid on MGA plates. These plates contained 2% glucose and Vogel Bonner "E" concentrate (9). Plates were incubated upside down in the dark at 37°C for 48 hours. Plates were prepared in triplicate, and the average revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control). The spontaneous reversion rate (with and without S-9) was monitored by averaging the counts from two determinations run simultaneously with the test compound. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Maron and Ames (6). Sterility and strain verification controls were run concurrently. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The integrity of the different *Salmonella* strains used in the assay was verified by the following standard tests:

- Lack of growth (inhibition) in the presence of crystal violet which indicates that the prerequisite alteration of the lipopolysaccharide layer (LP) of the cell wall is present.

- Growth in the presence of ampicillin-impregnated disks which indicates the presence of an ampicillin-resistant R Factor in all strains except TA1535, TA1537, and TA1538.

- Lack of growth (inhibition) following exposure to ultraviolet light which indicates the absence of the DNA excision-repair mechanism (all strains except TA102).

Six known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. Each strain must be tested with at least one positive control but may be tested with several. These compounds, benzo[a]pyrene (lot 18C-0378), 2-aminofluorene (lot 0-1547), 2-aminoanthracene (lot 020797), mitomycin-C (lot 0-[illegible]-065b), N-methyl-N'-nitro-N-nitrosoguanidine (lot 127C-

0342), and 4-nitroquinoline-*n*-oxide (lot 84F-0572), were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

Data Interpretation

According to Brusick (10), a compound is considered mutagenic if a positive dose response (correlated dose response) over three dose concentrations is achieved with at least the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count for the tester strains TA98 and TA100, or three times the spontaneous colony count for strains TA1535, TA1537, and TA1538. A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

Maron and Ames (6) consider a compound mutagenic in tester strains TA97 and TA102 if a correlated dose response over three concentrations is achieved with the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count.

Deviations from the Protocol/SOP

A 72-hour rather than a 48-hour incubation period was used for strain TA102 only. This gave the colonies an additional 24 hours to grow thus enabling all revertant colonies to be detected with the colony counter (Maron 1985, personal communication). Colony counts for all other strains were recorded after 48 hours.

Storage of the Raw Data and Final Report

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

On 16 May 1986, the toxicity of PHYSOSTIGMINE SALICYLATE was determined (Table 1). For this experiment all sterility, strain verification and negative controls were normal (Table 1). Exposure of the tester strain (TA100) to the two highest doses showed a decrease in the number of macrocolonies, and an

TABLE 1: TOXICITY LEVEL DETERMINATION FOR PHYSOSTIGMINE SALICYLATE

GLP STUDY NUMBER 87001

TOXICITY DETERMINATION REVERTANT PLATE COUNT (TA100)

CONCENTRATION	MEAN	±1SD	BACKGROUND LAWN*
START NEGATIVE CONTROL	76	4.7	NL
5.0 mg/plate	0	-	ST
1.0 mg/plate	21	7.6	ST
0.2 mg/plate	84	10.6	NL
0.04 mg/plate	80	10.4	NL
0.008 mg/plate	86	7.8	NL
0.0016 mg/plate	71	7.0	NL
END NEGATIVE CONTROL	91	7.2	NL

STRAIN VERIFICATION FOR TOXICITY DETERMINATION

	TA100*
HISTIDINE REQUIREMENT	NG
AMPICILLIN RESISTANCE	G
UV	NG
CRYSTAL VIOLET SENSITIVITY	NG
STERILITY CONTROL	NG

STERILITY CONTROL FOR TOXICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

*NL=Normal Lawn, G=Growth, NG=No Growth, ST=Slight Toxicity

observable reduction in the density of the background lawn, indicating chemical toxicity. Therefore, the highest dose selected for the mutagenicity test was 0.2 mg/plate. Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 13-15 August 1986 (Table 2). PHYSOSTIGMINE SALICYLATE did not induce an appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 3). A tabular presentation of the raw data is included in Appendix B.

DISCUSSION

Certain test criteria must be satisfied before an Ames Test can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, alterations in the LP layer, and deficiency in DNA excision-repair (except TA102). Second, the *Salmonella* strains must be susceptible to mutation by known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on formation of macrocolonies and microcolonies. If these tests are performed and expected data are obtained, then the results of an Ames Test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, PHYSOSTIGMINE SALICYLATE was evaluated in the Ames Test. Criteria for a positive response include both a correlated dose response over three dose concentrations, and a revertant colony count at least two times (TA97, TA98, TA100, TA102) (5,10) or three times (TA1535, TA1537, TA1538) (6,8) the spontaneous revertant colony count. PHYSOSTIGMINE SALICYLATE did not induce the requisite dose-response relationship or the increase in revertant colony counts necessary for a positive response. Thus, the results of this test indicate that PHYSOSTIGMINE SALICYLATE is not mutagenic when evaluated in the Ames Test.

CONCLUSION

PHYSOSTIGMINE SALICYLATE was evaluated for mutagenic potential in the Ames Test, in both the presence and the absence of metabolic activation, and did not induce a positive mutagenic response under conditions of this study.

TABLE 2: STRAIN VERIFICATION AND STERILITY TESTING FOR THE MUTAGENICITY DETERMINATION OF PHYSOSTIGMINE SALICYLATE

GLP STUDY NUMBER 87001

STRAIN VERIFICATION

	OBSERVATIONS*				
STRAIN	HISTIDINE REQUIREMENT	AMPICILLIN RESISTANCE	UV REPAIR	CRYSTAL VIOLET	STERILITY CONTROL
TA97	NG	G	NG	NG	NG
TA98	NG	G	NG	NG	NG
TA100	NG	G	NG	NG	NG
TA102	NG	G	G	NG	NG
TA1535	NG	NG	NG	NG	NG
TA1537	NG	NG	NG	NG	NG
TA1538	NG	NG	NG	NG	NG

STERILITY CONTROL FOR MUTAGENICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG
S-9	NG

*G = Growth, NG = No Growth

TABLE 3: Mutagenicity Assay for PHYSOSTIGMINE SALICYLATE (TW73)*

COMPOUND†	DOSE/PLATE	TA97	TA98	TA100	TA102
		WITHOUT S-9			
NEG CONTROL	0.0 mg	50 (21.6)	22 (3.4)	88 (7.5)	29 (7.2)
MITO-C	0.5 µg	-	-	-	96 (8.1)
MNNG	2.0 µg	-	-	94 (8.1)	-
MNNG	20.0 µg	-	-	-	-
NQNO	2.0 µg	237 (34.3)	226 (30.0)	937 (118.5)	-
TW73	0.2 mg	49 (10.2)	23 (4.7)	124 (5.9)	24 (3.2)
TW73	0.04 mg	74 (10.6)	23 (7.4)	122 (13.1)	38 (2.6)
TW73	0.008 mg	45 (6.8)	19 (3.8)	102 (11.9)	25 (3.0)
TW73	0.0016 mg	77 (7.8)	24 (6.8)	97 (7.8)	40 (4.2)
TW73	0.00032 ng	71 (16.2)	15 (6.4)	89 (3.6)	40 (6.7)
TW73	0.000064 mg	65 (30.2)	22 (4.5)	82 (14.7)	37 (11.0)
		WITH S-9			
NEG CONTROL	0.0 mg	56 (6.9)	37 (8.5)	89 (7.0)	57 (10.9)
AA	2.0 µg	-	742 (29.7)	948 (44.5)	-
AF	2.0 µg	276 (29.7)	678 (126.8)	593 (28.0)	-
BP	2.0 µg	184 (6.9)	122 (1.2)	288 (31.6)	-
TW73	0.2 mg	15 (8.6)	36 (4.4)	97 (3.0)	42 (13.4)
TW73	0.04 mg	65 (11.3)	40 (12.4)	97 (4.9)	88 (8.4)
TW73	0.008 mg	50 (3.8)	38 (10.7)	89 (7.8)	54 (5.5)
TW73	0.0016 mg	98 (6.1)	45 (4.0)	94 (11.4)	54 (13.6)
TW73	0.00032 mg	84 (14.2)	46 (12.5)	103 (7.4)	65 (8.7)
TW73	0.000064 mg	57 (11.3)	39 (7.5)	94 (2.1)	42 (8.1)

*Values represent the mean number of revertants/plate (± standard deviation)
†MITO-C=mitomycin-C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroguinoline-*n*-oxide, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

TABLE 3 (cont.): Mutagenicity Assay for PHYSOSTIGMINE SALICYLATE (TW73)*

COMPOUND†	DOSE/PLATE	TA1535	TA1537	TA1538
		WITHOUT S-9		
NEG CONTROL	0.0 mg	28 (4.6)	5 (2.4)	11 (2.7)
MNNG	20.0 µg	38 (6.4)	-	-
TW73	0.2 mg	28 (1.7)	8 (4.7)	14 (3.0)
TW73	0.04 mg	34 (2.5)	5 (1.5)	15 (1.5)
TW73	0.008 mg	29 (3.5)	12 (1.5)	12 (3.6)
TW73	0.0016 mg	34 (0.6)	7 (1.5)	15 (4.9)
TW73	0.00032 mg	30 (5.0)	5 (2.1)	7 (3.5)
TW73	0.000064 mg	39(11.4)	9 (6.6)	12 (1.7)
		WITH S-9		
NEG CONTROL	0.0 mg	22 (5.0)	9 (2.4)	29 (8.2)
AA	2.0 µg	-	20(10.5)	78(60.1)
AF	2.0 µg	-	-	807(77.7)
BP	2.0 µg	-	40 (0)	89 (4.9)
TW73	0.2 mg	35 (3.6)	10 (1.0)	40 (4.4)
TW73	0.04 mg	33 (5.5)	13 (4.5)	52 (6.5)
TW73	0.008 mg	22 (1.2)	11 (3.2)	30 (5.6)
TW73	0.0016 mg	34 (4.0)	10 (2.3)	29 (4.6)
TW73	0.00032 mg	27 (7.9)	14 (4.9)	22 (2.5)
TW73	0.000064 mg	26 (5.1)	10 (1.5)	27(10.1)

*Values represent the mean number of revertants/plate (± standard deviation
†MITO-C=mitomycin-C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-*n*-oxide, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

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APPENDICES

APPENDIX A: Chemical Data

Chemical Name: Physostigmine salicylate

Other Names: Eserine salicylate; Physostigmine, 2-hydroxybenzoate; 1, 2, 3, 3a, 8, 8a-Hexahydro-1, 3a, 8-trimethylpyrrolo[2,3-b]indol-5-ol methylcarbamate (ester), (3aS-cis)-, mono (2-hydroxybenzoate) (salt)

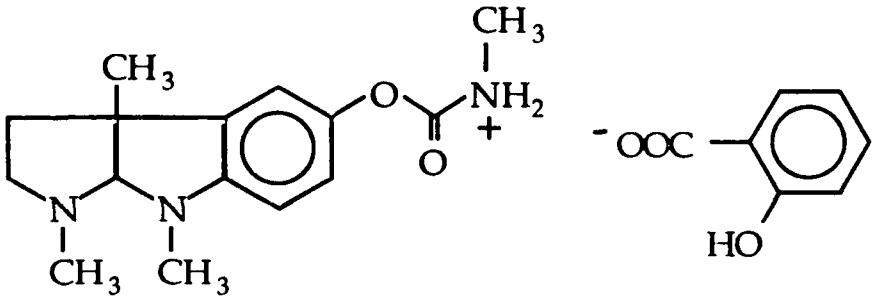
Lot Number: BL25591

Chemical Abstracts Service Registry Number: 57-64-7

LAIR Code: TW73

WRAIR Code: WR 6570AM

Chemical Structure:



Molecular Formula: $C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$

Molecular Weight: 413.47

Analytical Data:

The test compound was analyzed by the sponsors and the identity confirmed by UV and IR spectroscopy, high pressure liquid chromatography, mass spectrometry and elemental analysis.[1] Based on HPLC analysis of this test compound in comparison with the USP physostigmine salicylate reference standard, lot BL25591 contains 66.7% (100.1% of theory) physostigmine base and 33.7% (100.8% of theory) salicylic acid or 100.4% physostigmine salicylate.[1]

HPLC analysis of physostigmine salicylate in this lab was performed using a Hewlett-Packard 1090 HPLC system equipped with a diode array detector. The compound was chromatographed under the following conditions: silica

APPENDIX A (cont.): Chemical Data

column (4.6 x 100 mm, Brownlee Labs, Inc.); mobile phase, 15% acetonitrile/buffer (0.01M Na_2HPO_4 with 0.0025M tetramethylammonium chloride); flow rate, 1.5 ml/min; wavelength monitored, 210 nm. The compound eluted as two peaks with retention times of 0.9 min (salicylic acid), and 3.9 min (physostigmine).[2]

IR (KBr): 3320(broad), 2964, 2325, 1744, 1629, 1594, 1485, 1460, 1383, 1326, 1245, 1203, 1184, 1151, 1140, 1087, 1006, 993, 944, 860, 807, 754, 704, 667, 382 cm^{-1}.[3] The IR spectrum was identical to that provided by the sponsors[1].

Source: Bill Ellis
Division of Experimental Therapeutics
Walter Reed Army Institute of Research
Washington, DC
Requested by LTC Jurgen von Bredow, PhD, MSC

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[1]Matsumori E, Benitez A, and Lim P. Assay of physostigmine salicylate, WR-6570AM, BL25591. Menlo Park, CA: SRI International, 4 November 1986; Report no. 553.

[2]Wheeler CR. Toxicity testing of antidotes of chemical warfare agents. Laboratory notebook #85-12-024.1, pp 2-11. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3]Wheeler CR. Toxicity testing of antidotes of chemical warfare agents. Laboratory notebook #85-12-024.3, pp 10-11. Letterman Army Institute of Research, Presidio of San Francisco, CA.

APPENDIX B: Individual Plate Scores

PHYSOSTIGMINE SALICYLATE (TW73)

TOXICITY DETERMINATION WITH TA100

DOSE/PLATE	5.0 mg	1.0 mg	0.2 mg	0.04 mg
PLATE 1	0	30	86	88
PLATE 2	0	16	73	83
PLATE 3	0	18	94	68
background lawn*	ST	ST	NL	NL

DOSE/PLATE	0.008 mg	0.0016 mg	NEG START	NEG END
PLATE 1	88	76	81	99
PLATE 2	91	65	74	86
PLATE 3	79	-	72	87
background lawn*	NL	NL	NL	NL

* ST=Slight Toxicity, NL=Normal Lawn

APPENDIX B (cont.): Individual Plate Scores

PHYSOSTIGMINE SALICYLATE (TW73)

NEGATIVE CONTROL DATA

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
				WITHOUT S-9				
NEG CONTROL (START RUN)	0.0 mg	74	22	96	41	35	7	8
		64	28	99	29	32	9	12
		67	22	84	25	23	4	15
NEG CONTROL (END RUN)	0.0 mg	38	20	84	28	24	3	11
		21	18	82	29	28	4	9
		33	24	83	19	27	3	-
				WITH S-9				
NEG CONTROL (START RUN)	0.0 mg	55	43	90	77	23	5	44
		57	47	95	59	27	10	26
		62	37	91	56	24	8	33
NEG CONTROL (END RUN)	0.0 mg	46	36	82	53	26	7	26
		50	22	95	44	21	11	24
		64	37	78	54	13	11	22

APPENDIX B (cont.): Individual Plate Scores

POSITIVE CONTROL DATA
PHYSOSTIGMINE SALICYLATE (TW73)

COMPOUND†	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
AA	2.0 µg		769	996			19	146
			746	941			10	56
			710	908			31	32
AF	2.0 µg	254	532	564				852
		265	746	594				851
		310	757	620				717
BP	2.0 µg	176	121	298			40	87
		188	123	314			40	86
		189	121	253			40	95
MITO-C	0.5 µg				91			
					105			
					91			
MNNG	2.0 µg			85				
				95				
				101				
MNNG	20.0 µg					45		
						33		
						35		
NQNO	2.0 µg	232	235	1073				
		206	251	856				
		274	193	882				

†MITO-C=mitomycin-C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

APPENDIX B (cont.): Individual Plate Scores

PHYSOSTIGMINE SALICYLATE (TW73)

MUTAGENICITY DATA WITHOUT S-9

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
TW73	0.2 mg	42	21	122	25	29	13	17
		61	28	131	26	29	6	14
		45	19	120	20	26	4	11
TW73	0.04 mg	62	31	124	39	37	4	15
		82	20	134	35	32	7	16
		78	17	108	40	34	5	13
TW73	0.008 mg	40	23	92	22	26	14	11
		43	17	115	25	29	12	9
		53	16	98	28	33	11	16
TW73	0.0016 mg	83	16	101	35	34	7	13
		68	26	102	43	33	8	21
		79	29	88	41	34	5	12
TW73	0.00032 mg	61	8	85	44	25	7	3
		63	18	90	43	35	3	7
		90	20	92	32	30	4	10
TW73	0.000064 mg	97	22	93	33	26	2	13
		37	27	65	28	42	10	10
		61	18	87	49	48	15	13

APPENDIX B (cont.): Individual Plate Scores

PHYSOSTIGMINE SALICYLATE (TW73)

MUTAGENICITY DATA WITH S-9

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
TW73	0.2 mg	7	34	100	27	34	9	45
		13	33	94	48	39	10	38
		24	41	97	52	32	11	37
TW73	0.04 mg	58	47	95	83	33	17	58
		78	48	103	84	27	13	52
		59	26	94	98	38	8	45
TW73	0.008 mg	48	40	95	60	21	9	31
		47	47	91	50	23	15	35
		54	26	80	51	21	10	24
TW73	0.0016 mg	95	47	102	49	35	11	32
		94	47	81	43	30	7	24
		105	40	99	69	38	11	32
TW73	0.00032 mg	69	37	100	58	18	17	20
		87	40	97	63	33	16	25
		97	60	111	75	30	8	22
TW73	0.000064 mg	51	32	92	47	30	11	38
		70	47	96	33	20	10	25
		50	39	95	47	27	8	18

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Head, Biological Sciences Division
OFFICE OF NAVAL RESEARCH
800 North Quincy Street
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